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Micromegakaryocytes in a Patient With Partial Deletion of the Long Arm of Chromosome 11 [del(11)(q24.2qter)] and Chronic Thrombocytopenic Purpura

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Thrombocytopenia or pancytopenia is frequently reported in patients with partial 11q deletion but there are no reports on bone marrow morphology of these patients. We report on a patient with partial deletion of the long arm of chromosome 11 [del(11)(q24.2qter)] and its classical clinical manifestations including chronic thrombocytopenic purpura in whom micromegakaryocytes were found in the bone marrow aspirate. This is the first report of the presence of micromegakaryocytes in the bone marrow of a patient with 11q deletion. Accurate examination of the bone marrow of other patients with the 11q deletion may clarify whether the observation of micromegakaryocytes is common in these patients. Micromegakaryocytes may indicate a defect of development. Two genes for two DNA binding proteins that are likely to be involved in hematopoiesis map in the 11q region: Ets-1, that maps to 11q24, close to D11S912, and the nuclear-factor-related-kB gene that maps to 11q24–q25. It is possible that these genes, when present in only one copy, result in thrombocytopenia or pancytopenia as observed in this patient. © 1996 Wiley-Liss, Inc.

KEY WORDS: chromosome 11q deletion, micromegakaryocytes

INTRODUCTION

Partial deletion of the distal end of the long arm of chromosome 11 (Jacobsen syndrome) has been reported in about 40 subjects [Wardinsky et al., 1990; Neavel et al., 1994].

Clinical manifestations include postnatal growth retardation, mild psychomotor retardation, trigonocephaly, minor facial anomalies, cardiac defects, digit anomalies, and thrombocytopenia or pancytopenia. None of these traits is invariably present.

Thrombocytopenia or pancytopenia has been detected in about one-half of these patients regardless of the extent of the deletion. These findings can resolve in some patients and have been observed intermittently in other patients [Penny et al., 1995].

There is only one report of bone marrow morphology in the cases described by other authors with no mention of the size of megakaryocytes [Levine et al., 1980; Penny et al., 1995].

We report on a patient with 11q deletion and the classical manifestations of this abnormality including chronic thrombocytopenic purpura in whom micromegakaryocytes could be shown in the bone marrow aspirate.

CLINICAL REPORT

A 3-year-old boy was admitted to our Division because of diagnostic evaluation for chronic thrombocytopenia.

Low platelet count (65,000–85,000/mmc) had been observed from birth. Only one episode of pancytopenia had been observed during the follow-up.

Family history was unremarkable. Parents were healthy and nonconsanguineous. β-Thalassemic trait was present in the mother. The father was 34 and the mother 33 years old at time of the patient's birth. The mother had had two previous miscarriages and one older normal daughter.

The patient was born at 34 weeks of gestation by cesarean section because of premature rupture of the membranes. His birth weight was 1860 g (10th centile)

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and length 43 cm (10th centile); head circumference (OFC) was 31 cm (25th centile).

Physical examination showed high forehead, hypertelorism, broad flat nasal bridge, down slant of palpebral fissures, short nose, "carp mouth," apparently low-set ears (Fig. 1), inguinal hernia with cryptorchidism, syndactyly of toes 2–3 bilaterally, and small ecchymoses on the limbs. There was no organomegaly; lungs and heart were normal.

A complete blood count showed white blood cell count, 7600/mmc (differential count: N 46%, E 4%, L 50%); hemoglobin values 9.8 g/dl; red blood cell count 5,100,000/mmc; mean corpuscular volume (MCV) 61 fl; reticulocyte count, 45,900/mmc, and a platelet count of 88,000/mmc.

Peripheral blood smear showed slight anisopoikilocytosis of red cells. Platelets appeared to be normal in size and morphology. Neutrophil inclusions (May-Hegglins anomaly) were absent. Hemoglobin electrophoresis showed 1% of HbF and 4.50% of HbA2. Iron status and levels of serum immunoglobulins and of C3c and C4 factor of complement were normal. Platelet antibodies and antinuclear antibodies were absent.

Chromosome analysis performed because of the unusual physical appearance of the patient showed a partial deletion of the long arm of chromosome 11 [del(11)(q24.2qter)].

This case was included in a collaborative study on distal 11q deletion (patient 12) [Penny et al., 1995].

Bone marrow aspirate, prepared with Wright–Giemsa stain, showed a normal myeloerythroid ratio with normal cell morphology. A slight increase of number of megakaryocytes was present. The size of megakaryocytes was reduced in all the stages of maturation with increase of the number of small mononuclear cells (Fig. 2a and b).

Size, maturation, and ploidy of the patient's megakaryocytes were quantified in Wright–Giemsa and Feulgen-stained bone marrow smears [Levine et al., 1980]. The size of megakaryocytes was determined at 1000× magnification with the aid of an eyepiece micrometer. As controls we used bone marrow preparations from 3 normal children (two with β-thalassemic-trait as in our patient) and from 3 children affected by idiopathic thrombocytopenic purpura (ITP) whose bone marrow aspirate had been performed during diagnostic procedures (Table I). Two hundred megakaryocytes were examined on each patient. Statistical analysis for megakaryocyte size differences in the 3 groups was done by the Mann–Whitney nonparametric test.

Patient's megakaryocyte size ranged from 6 to 36 μm (mean ± SD: 16.24 ± 7.4) and was significantly reduced with respect to that of 3 normal children (mean ± SD: 33.6 ± 15.2 μm) ($P < 0.02$) and of 3 children affected by ITP (mean ± SD: 69.2 ± 12.5 μm) ($P < 0.01$). Low megakaryocyte ploidyzation and block of maturation were also observed (Table I).

Patient serum level of IL-6, a thrombocytopoietic factor that promotes the maturation of megakaryocytes, detected by an enzyme immunoassay kit (Cytokit TM6, Genzyme), was 1.802 ng/ml (normal subjects that we

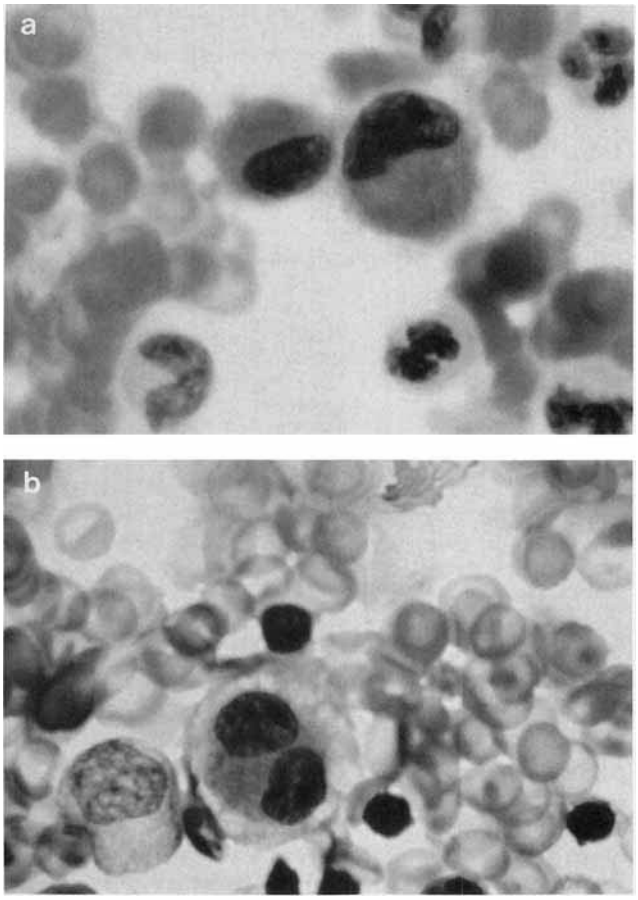

Fig. 2. Patient's Wright–Giemsa stained bone marrow smear (×2,000) showing two small stage IV megakaryocytes (11 and 14 μm) (**a**) and a small stage II vacuolated megakaryocyte (20 μm) (**b**). Their sizes are about the same as the myeloid elements near them. Megakaryocytes were easily identified by their cytoplasmatic staining.

Fig. 1. Facial appearance of the patient.

TABLE I. Maturational Stage, Size, and Ploidy of Megakaryocytes in the Patient and Controls

	Patient	Controls (n = 3)	ITP (n = 3)
Size (μm: mean ± SD)	16.24 ± 7.4	33.6 ± 15.2	69.2 ± 12.5
Maturational stages (%)			
I	18.3	30.2	3.8
II	22.0	13.0	11.2
III	43.7	29.8	70.0
IV	16.0	27.0	15.0
Ploidy frequencies (%)			
4 N	35.3	15.8	1.0
8 N	36.7	21.8	4.5
16 N	23.4	27.1	14.6
32 N	4.6	25.2	25.6
64 N	—	10.1	31.5
128 N	—	—	20.8
256 N	—	—	2.0

used as controls: 1.540 ± 0.267 (ng/ml) [Gangarossa et al., 1995]. We have not examined thrombopoietin activity.

DISCUSSION

We present a case of congenital thrombocytopenic purpura with micromegakaryocytes in a patient with constitutional del11q and the accompanying clinical phenotype.

Micromegakaryocytes were observed after the submission of the collaborative study [Penny et al., 1995]. in which the patient was included.

Pancytopenia or thrombocytopenia are common findings in patients with 11q deletion [O'Hare et al., 1984; Küster et al., 1985; Wardinsky et al., 1990; Neavel and Soukup, 1994; Penny et al., 1995], but there is no report of micromegakaryocytes in the bone marrow of these patients.

Bone marrow analysis performed in one case of pancytopenia associated with del11q24.1qter showed hypocellularity with presence of megakaryocytes. The myeloid activity was reduced but with normal maturation and differentiation. Erythropoiesis was depressed but normoblastic [O'Hare et al., 1984].

In our case megakaryocytes with a very small size were present when compared to normal controls and to patients affected by ITP. Myeloid and erythroid elements were morphologically normal.

Micromegakaryocytes have been reported in the thrombocytopenia-absent radius (TAR) syndrome [de Alarcon et al., 1991], preleukemia, acute leukemia, chronic myelogenous leukemia, the myelodisplastic syndrome [Kuriyama et al., 1986], pseudo-Pelger–Huët abnormality [Jackson and Dahl, 1983], multiple myeloma, and refractory anemia [Smith et al., 1973]. Familial cases have also been reported [Olson et al., 1992].

Normally, megakaryocyte size is linked to maturation stage and to ploidy [Levine et al., 1982]. Micromegakaryocytes may indicate a defect of development. Several growth factors are involved in megakaryocytopoiesis, mainly IL-3, IL-6, IL-11, Kitligand, and c-mpl ligand, now identified as the thrombopoietin [de Sauvage et al., 1994; Debili et al., 1994; Lok et al., 1994; Kaushansky et al., 1994; Wendling et al., 1994]. In vivo c-mpl ligand stimulates platelet production by expanding megakaryocytes and their progenitor and by shifting the distribution of megakaryocyte ploidy to higher values [de Sauvage et al., 1994; Lok et al., 1994; Kaushansky et al., 1994; Wendling et al., 1994]. Anomaly of megakaryocyte development may be related to deficit of cytokine production or to specific receptor deficit on megakaryocytes.

Two genes that are likely to play some role in hematopoiesis are involved in distal 11q deletions: Ets-1 gene and the nuclear-factor-related-kB (NFRKB) gene.

Ets-1 is a component of the Ets family of DNA-binding proteins that maps to 11q24, close to D11S912 [Selleri et al., 1992] and is expressed only in hematopoietic tissue [Ben-David et al., 1991]. Ets proteins bind to a variety of transcriptional enhancers and promoters and may play a role in transcriptional regulation [MacLeod et al., 1992]. Ets-binding sites are found in regulatory regions of many genes expressed in hematopoietic cell including the IL-2 gene, the stromolysin gene, and the T-cell receptor α- and β-chain genes [MacLeod et al., 1992].

The NFRKB gene maps to 11q24–25 and is expressed preferentially in T and B cell [Adams et al., 1992]. The product of this gene in involved in IL-2 receptor-gene expression, which is a critical event in T-cell activation, and may have additional roles in hematopoietic differentiation.

The presence of only one copy of one or both of these genes in patients with 11q deletions may be responsible for abnormal hematopoietic differentiation and/or development and consequently for the pancytopenia or thrombocytopenia. The presence of micromegakaryocytes may be the morphologic aspect of defect of development.

Thrombocytopenia in our patient may be related to a defect of platelet production by micromegakaryocytes because platelet production seems to be related to the cytoplasmic volume of megakaryocytes [Levine et al., 1982; Wendling et al., 1994].

As there is only limited information about bone marrow morphology in patients with del11q we do not know if this is a common finding in these patients. Further-

more we have no information about the chromosome status in patients with micromegakaryocytes.

Future reports of fully documented patients will be of value in the clarification of this issue.

NOTE ADDED IN PROOF

After this paper was submitted a report concerning a similar observation has been published [Breton-Gorius J, Favier R, Guichard J, Cherif D, Berger R, Debili N, Vainchenker W, Douay L (1995): A new congenital dysmegakaryopoietic thrombocytopenia (Paris-Trousseau) associated with giant platelet α-granules and chromosome 11 deletion at 11q23. Blood 85 (April):1805–1814.] Furthermore we have observed another patient affected by chromosome 11q 24.2 deletion and chronic dysmegakaryopoietic thrombocytopenia with micromegakaryocytes.

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